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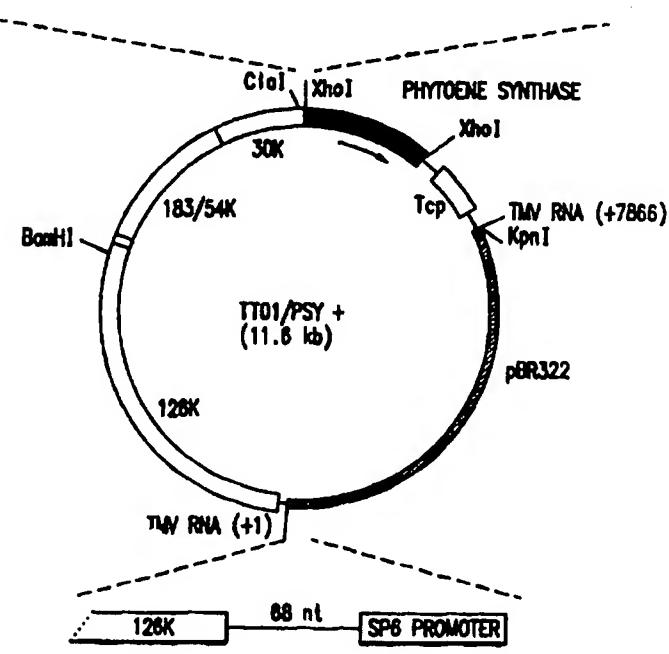
(54) Title: THE CYTOPLASMIC INHIBITION OF GENE EXPRESSION

(57) Abstract

One aspect of the invention is to provide novel genetic constructions for the expression of inhibitory RNA in the cytoplasm of eukaryotic cells. The inhibitory RNA may be an anti-sense RNA or a co-suppressor RNA. The genetic constructions of the invention are capable of replicating in the cytoplasm of a eukaryotic cell and comprise a promoter region in functional combination with an encoding polynucleotide. The genetic constructions may be designed so as to replicate in the cytoplasm of plant cells, yeast cells, and mammalian cells. When the eukaryotic cell of interest is a plant cell, the genetic construction is preferably derived from a plant RNA virus. Plant RNA virus derived genetic constructions may employ a plant virus subgenomic promoter, including subgenomic promoters from tobamoviruses in functional combination with the RNA encoding region. Another aspect of the invention is to provide cells comprising the genetic constructions of the invention and organism comprising a plurality of such cells. Another aspect of the invention is to provide methods of reducing the expression of a gene of interest in eukaryotic cells, i.e., methods of producing eukaryotic cells exhibiting reduced levels of expression of a gene of interest. The methods of the invention comprise the step of transfecting a cell with a genetic construction of the invention in which the RNA encoding region is specific for the gene of interest. Another aspect of the invention is to provide plant cells that produce elevated levels of the carotenoid phytoene. The elevated levels of phytoene are achieved by inhibiting the expression at the enzyme phytoene desaturase using the vectors of the invention.

.isp XbaI Start codon  
CTTAAATAACCTCGAGCTTAAAT ATC TCT GTT CCC TTC TGA CTT GTT TCT CCT TGT GAC  
Met Ser Val Ala Leu Leu Tyr Val Val Ser Pro Cys Asp

TRANSIT PEPTIDE OF PHYTOENE SYNTHASE  
GTC TCA AAT GCG ACA AGT TTC ARG GAA TCA GTC CGG GAG CGA AAC CGT  
Val Ser Asn Gly Thr Ser Phe Met Glu Ser Val Arg Glu Gly Asn Arg



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**INTERNATIONAL SEARCH REPORT**

International Application No PCT/US 95/06741
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**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6	C12N15/83	C12N15/11	C12N15/53	C12N15/52	C12N5/10
A01H5/00					

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VIRUS RESEARCH, vol. 32, no. 1, 1994 pages 57-67, POWERS, A.M., ET AL. 'INTRACELLULAR IMMUNIZATION OF MOSQUITO CELLS TO LACROSSE VIRUS USING A RECOMBINANT SINDBIS VIRUS VECTOR' see the whole document ---	1,2,9, 10,17, 18,31,32
X	WO,A,93 03161 (DONSON JON ;DAWSON WILLIAM O (US); GRANTHAM GEORGE L (US); TURPEN) 18 February 1993 see page 23, line 21 - line 25 see page 30, last paragraph - page 31 ---	1-38 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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20 December 1995

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Inte onal Application No  
PCT/US 95/06741

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 13994 (COMMW SCIENT IND RES ORG) 19 September 1991  see the whole document ---	1-6, 9-14, 17-22, 25-28, 31-36
X	EP,A,0 425 004 (AVEVE NV ;CLOVIS MATTON N V (BE); SOLVAY (BE)) 2 May 1991  see the whole document ---	1-5, 9-14, 17-22, 25-28, 31-36
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 92 (5). 1995. 1679-1683. KUMAGAI M H 'Cytoplasmic inhibition of carotenoid biosynthesis with virus-derived RNA.' see the whole document ---	1,2
A	WO,A,90 12107 (SALK INST BIOTECH IND) 18 October 1990 see page 13, line 30 - line 37 ---	1-38
A	NATURE, vol. 334, 14 July 1988 pages 179-182, HAYES, R.J., ET AL. 'Gene amplification and expression in plants by a replicating geminivirus vector' see page 182, last paragraph ---	1-38
A	THE PLANT JOURNAL, vol. 2, no. 3, 1992 pages 343-349, BRAMLEY, P., ET AL. 'BIOCHEMICAL CHARACTERIZATION OF TRANSGENIC TOMATO PLANTS IN WHICH CAROTENOID SYNTHESIS HAS BEEN INHIBITED THROUGH THE EXPRESSION OF ANTISENSE RNA TO PTOM5' see the whole document ---	
A	PLANT JOURNAL, vol. 4, no. 5, 1993 pages 833-840, MISAWA, N., ET AL. 'FUNCTIONAL EXPRESSION OF THE ERWINIA UREDOVORA CAROTENOID BIOSYNTHESIS GENE CRTL IN TRANSGENIC PLANTS SHOWING AN INCREASE OF BETA-CAROTENE BIOSYNTHESIS ACTIVITY AND RESISTANCE TO THE BLEACHING HERBICIDE NORFLURAZON' see the whole document ---	
2		-/-

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/06741

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,91 13078 (AMOCO CORP) 5 September 1991 see the whole document ----	
A	WO,A,91 09128 (ICI PLC) 27 June 1991 see the whole document -----	

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**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No  
PCT/US 95/06741

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9303161	18-02-93	US-A-	5316931	31-05-94
		AU-A-	3351193	02-03-93
		CA-A-	2114636	18-02-93
		EP-A-	0596979	18-05-94
		JP-T-	7503361	13-04-95
-----	-----	-----	-----	-----
WO-A-9113994	19-09-91	NONE		
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EP-A-0425004	02-05-91	NL-A-	8902452	01-05-91
		NL-A-	9001711	01-05-91
		CA-A-	2026703	04-04-91
		JP-A-	3280883	11-12-91
-----	-----	-----	-----	-----
WO-A-9012107	18-10-90	NONE		
-----	-----	-----	-----	-----
WO-A-9113078	05-09-91	EP-A-	0471056	19-02-92
		JP-T-	5504686	22-07-93
-----	-----	-----	-----	-----
WO-A-9109128	27-06-91	AU-B-	645534	20-01-94
		AU-B-	6893891	18-07-91
		EP-A-	0505405	30-09-92
		JP-T-	5502160	22-04-93
		US-A-	5304478	19-04-94
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